Figure 1a

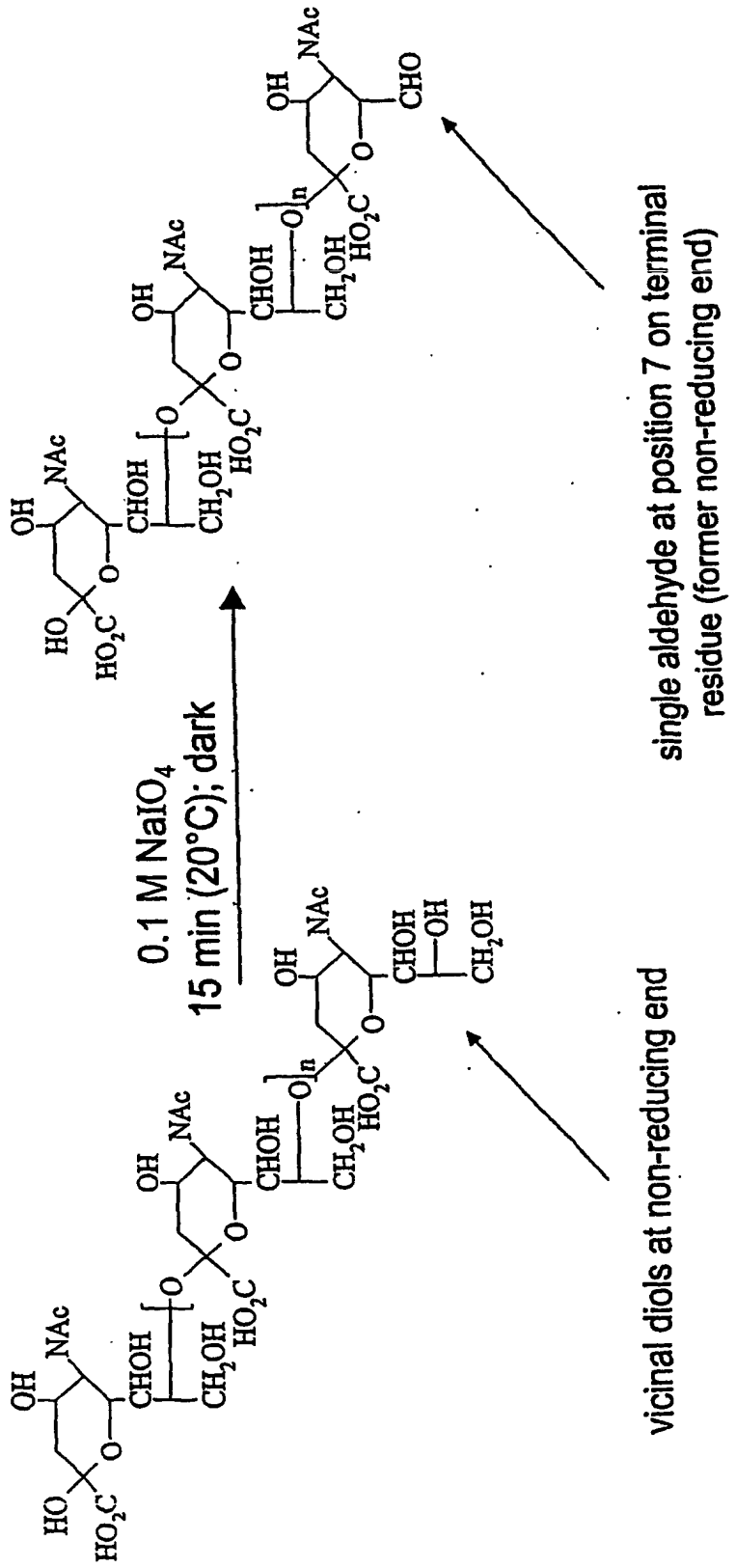

0.1 M NaIO4
15 min (20°C); dark
vicinal diols at non-reducing end
single aldehyde at position 7 on terminal residue (former non-reducing end)

Figure 1b

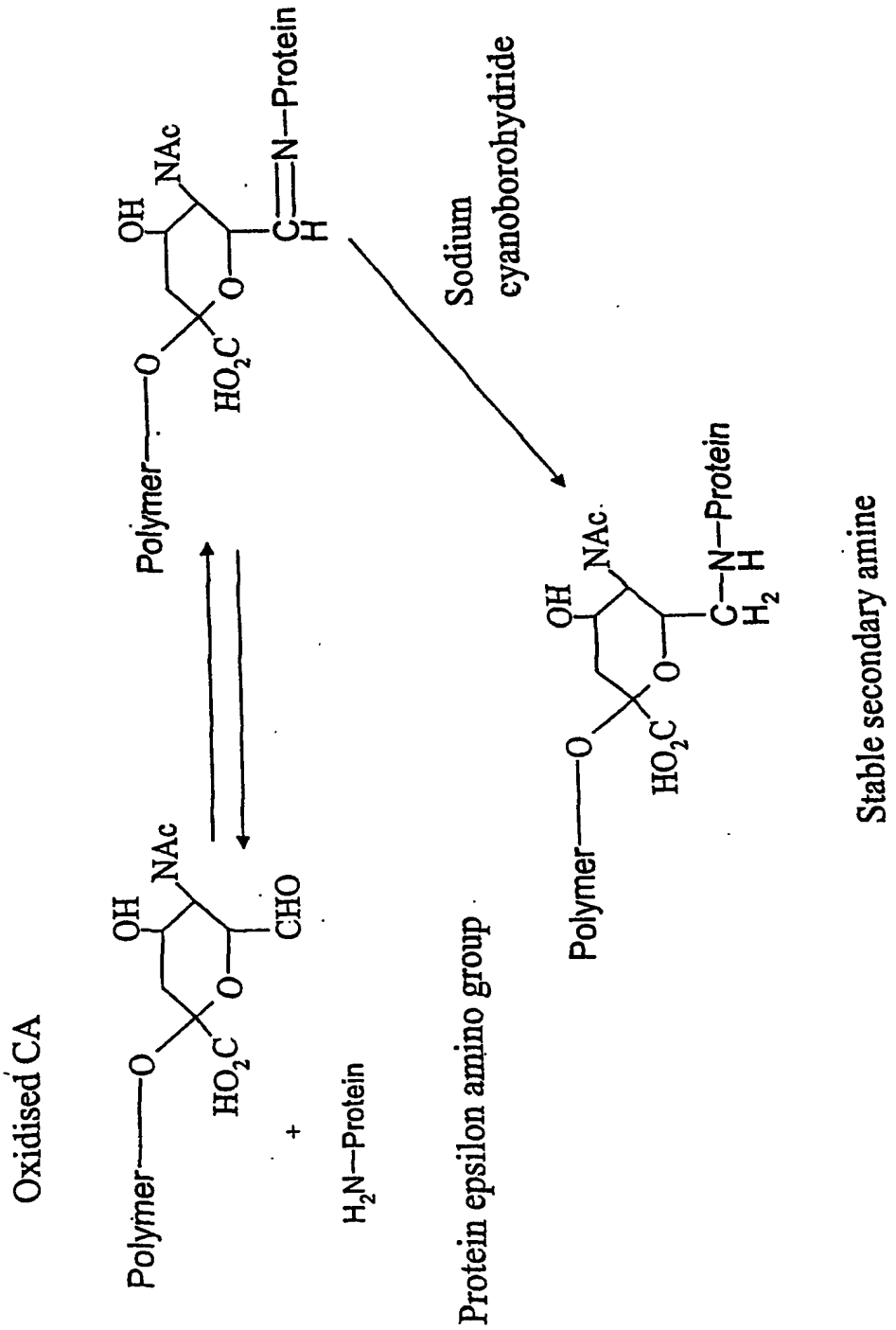
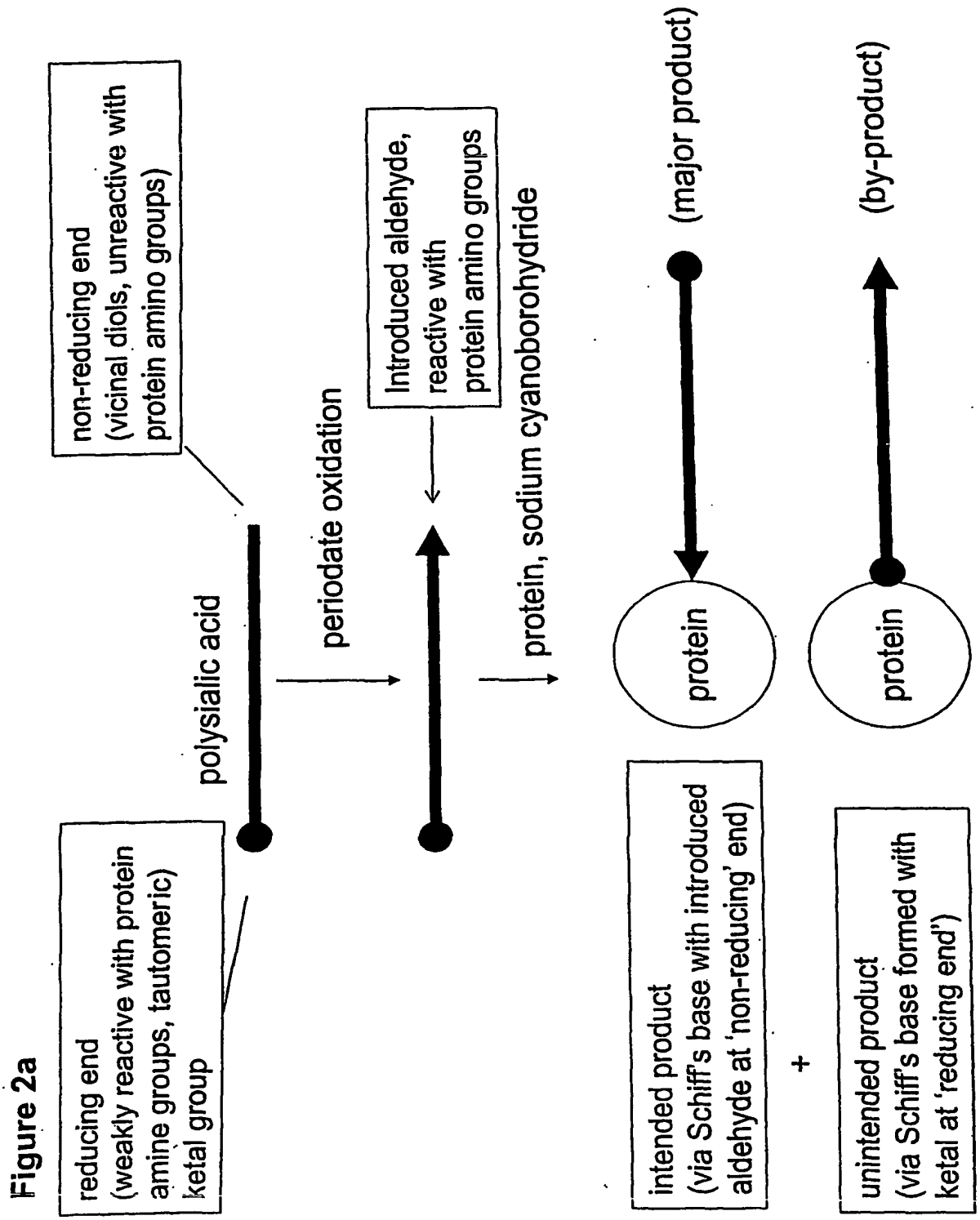

Oxidised CA
Polymer
OH
NAc
CHO
HO_2C
O
+
H_2N—Protein
Protein epsilon amino group
Sodium cyanoborohydride
Polymer
OH
NAc
C=N—Protein
H
HO_2C
O
Stable secondary amine
Polymer
OH
NAc
C—N—Protein
H_2 H
HO_2C
O

Figure 2a


reducing end
(weakly reactive with protein amine groups, tautomeric) ketal group
polysialic acid
non-reducing end
(vicinal diols, unreactive with protein amino groups)
periodate oxidation
Introduced aldehyde, reactive with protein amino groups
protein, sodium cyanoborohydride
protein
(major product)
protein
(by-product)
intended product
(via Schiff's base with introduced aldehyde at 'non-reducing' end)
+
unintended product
(via Schiff's base formed with ketal at 'reducing end')

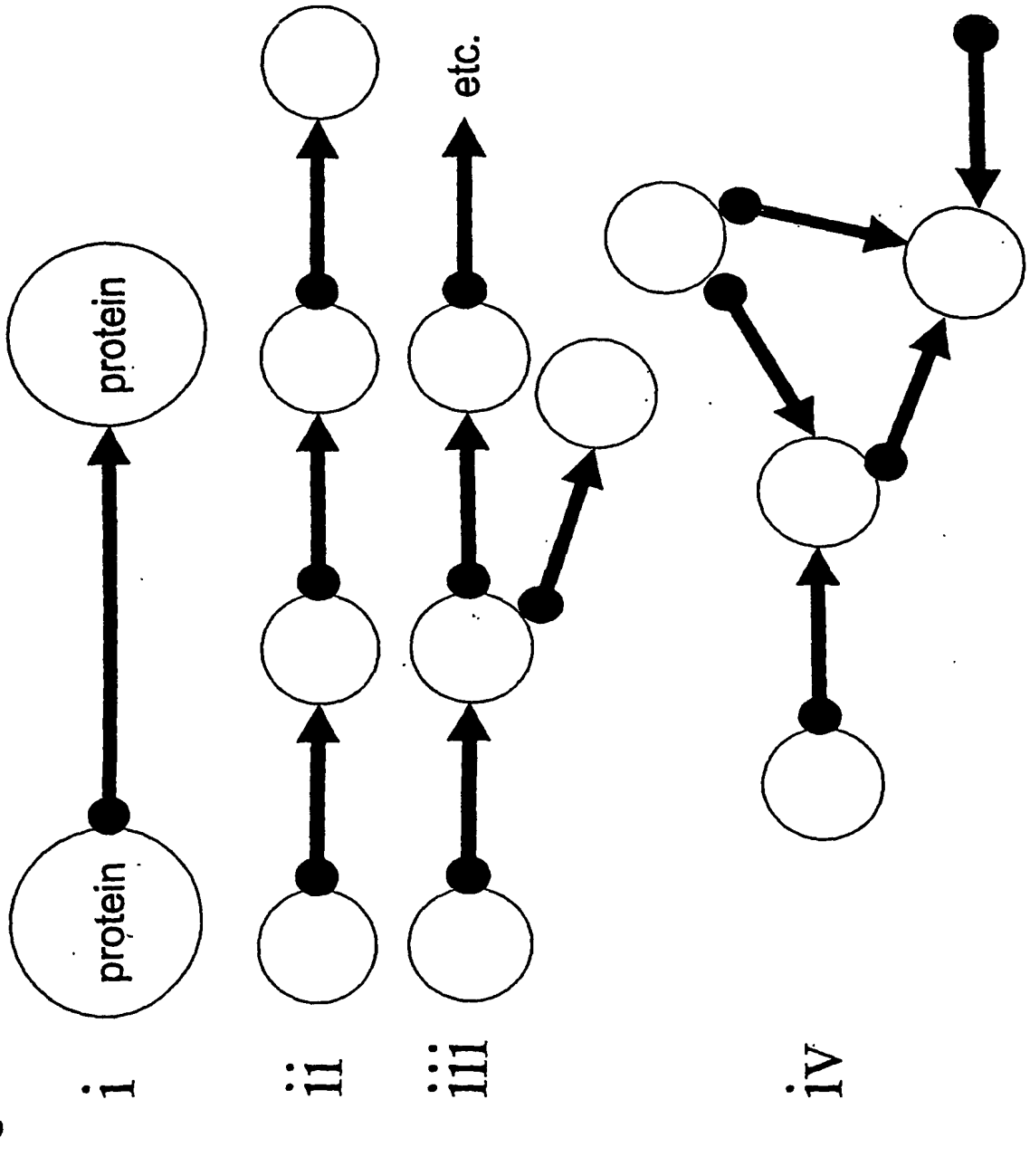
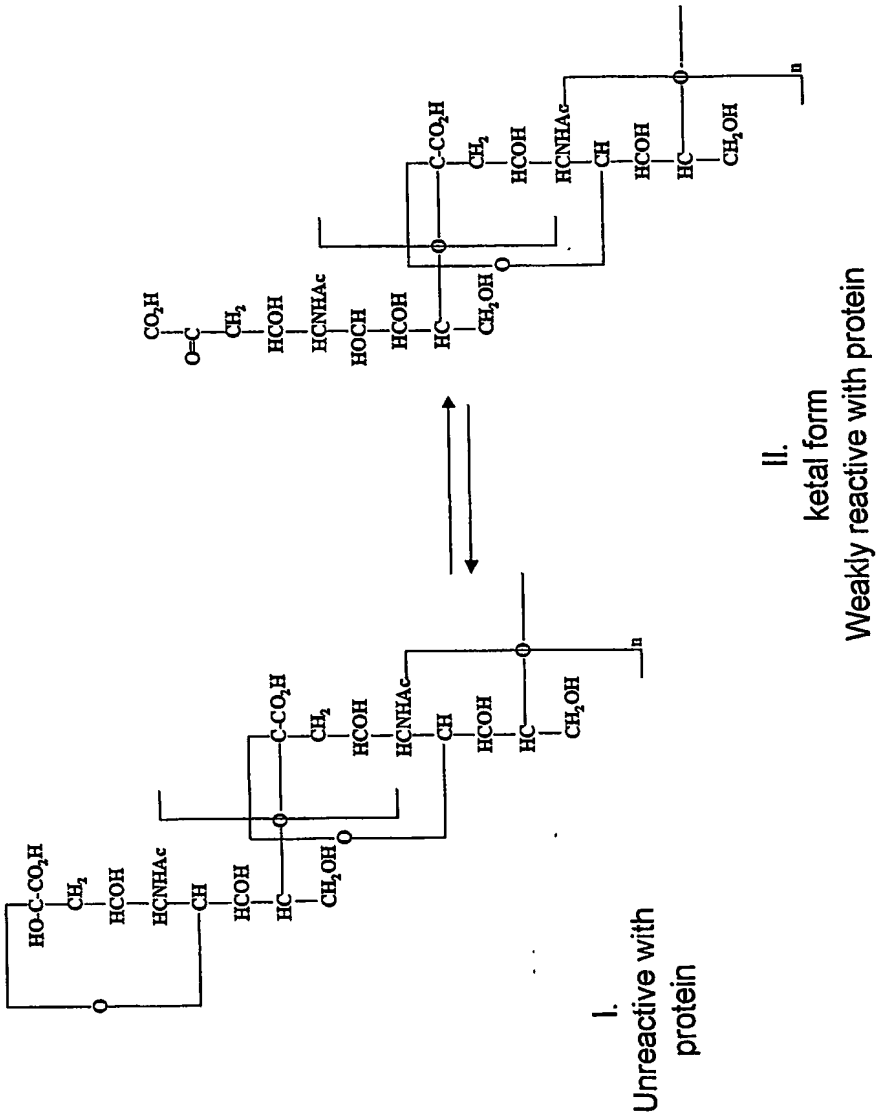


Figure 2b

Figure 3


I.
Unreactive with protein
II.
ketal form
Weakly reactive with protein

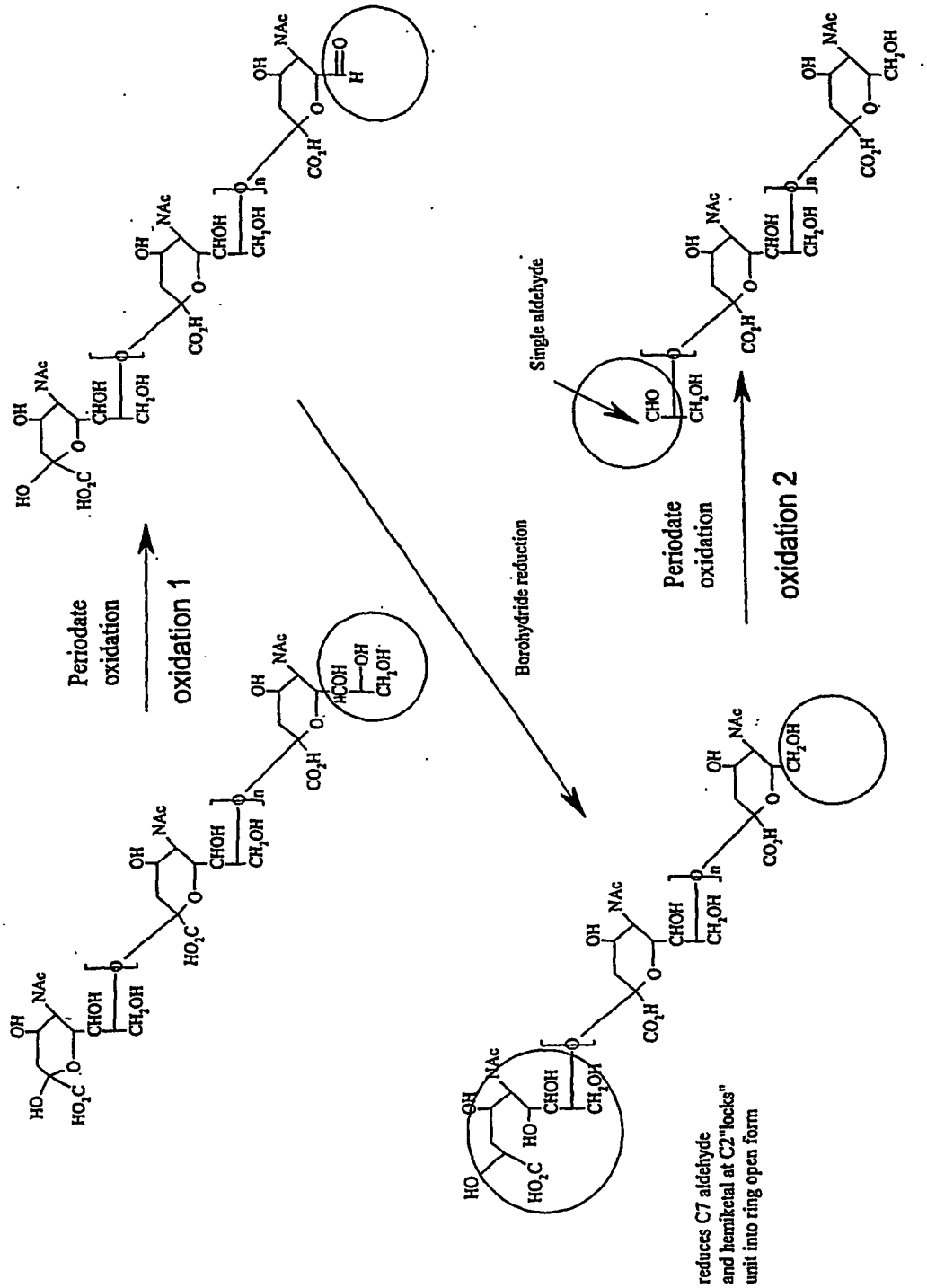

Periodate oxidation
oxidation 1
Borohydride reduction
Single aldehyde
Periodate oxidation
oxidation 2
reduces C7 aldehyde and hemiketal at C2 "locks" unit into ring open form

Figure 4a

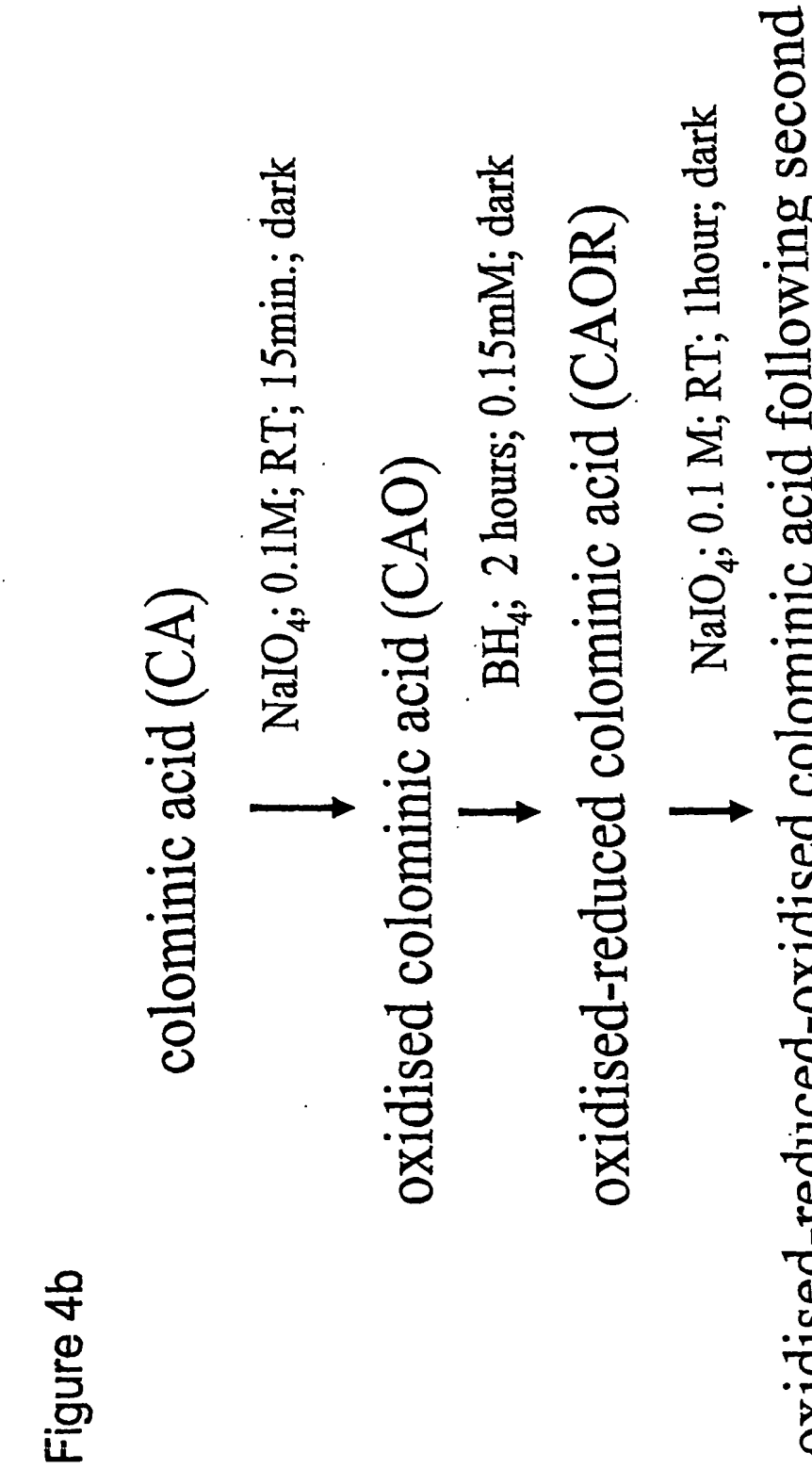
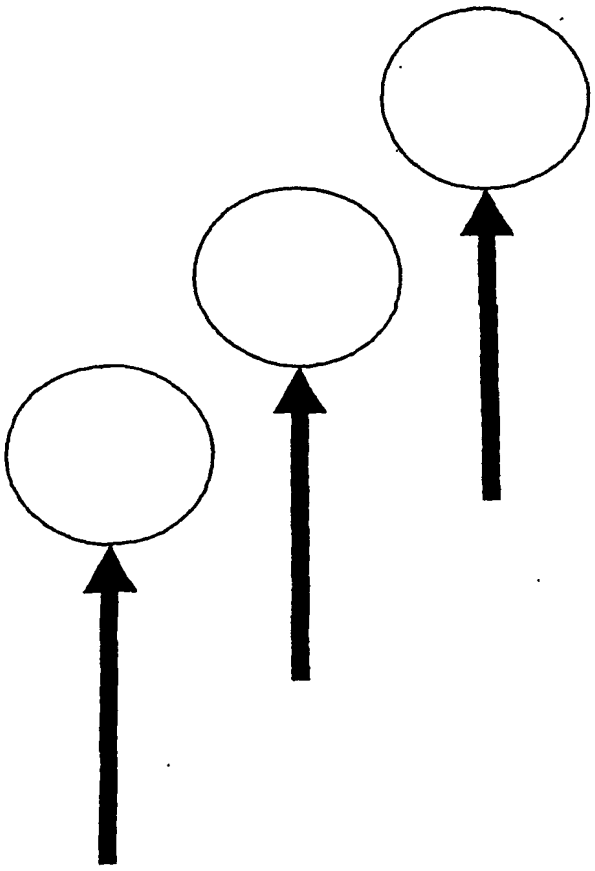

colominic acid (CA)
NaIO4; 0.1M; RT; 15min.; dark
oxidised colominic acid (CAO)
BH4; 2 hours; 0.15mM; dark
oxidised-reduced colominic acid (CAOR)
NaIO4; 0.1 M; RT; 1hour; dark
oxidised-reduced-oxidised colominic acid following second oxidation (CAORO)

Figure 4b

Figure 5



Monofunctional polysialic acid cannot form unintended by-products described for periodate-oxidised natural polysialic acid in Fig. 1

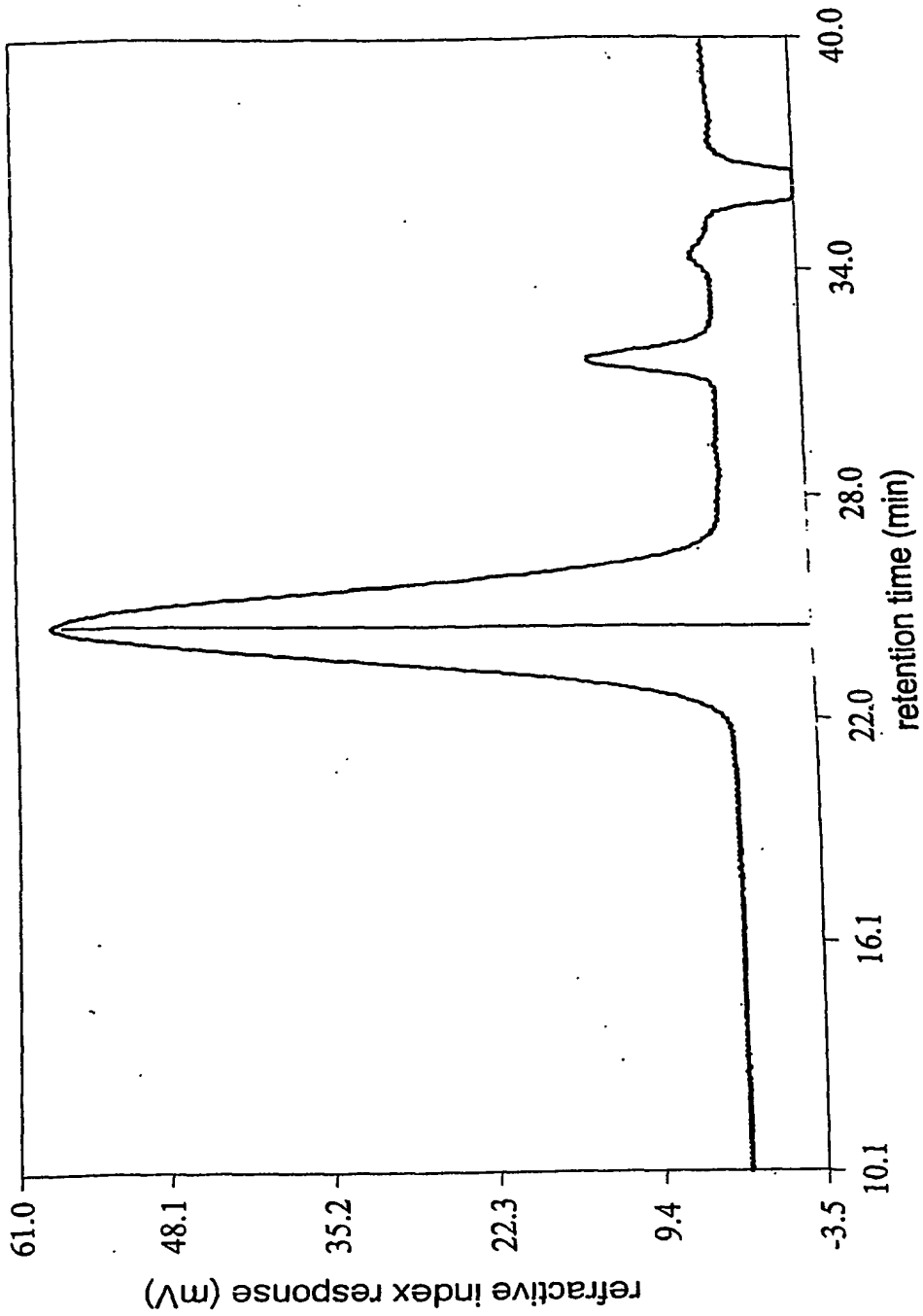

refractive index response (mV)
61.0
48.1
35.2
22.3
9.4
-3.5
10.1
16.1
22.0
28.0
34.0
40.0
retention time (min)

Figure 6a

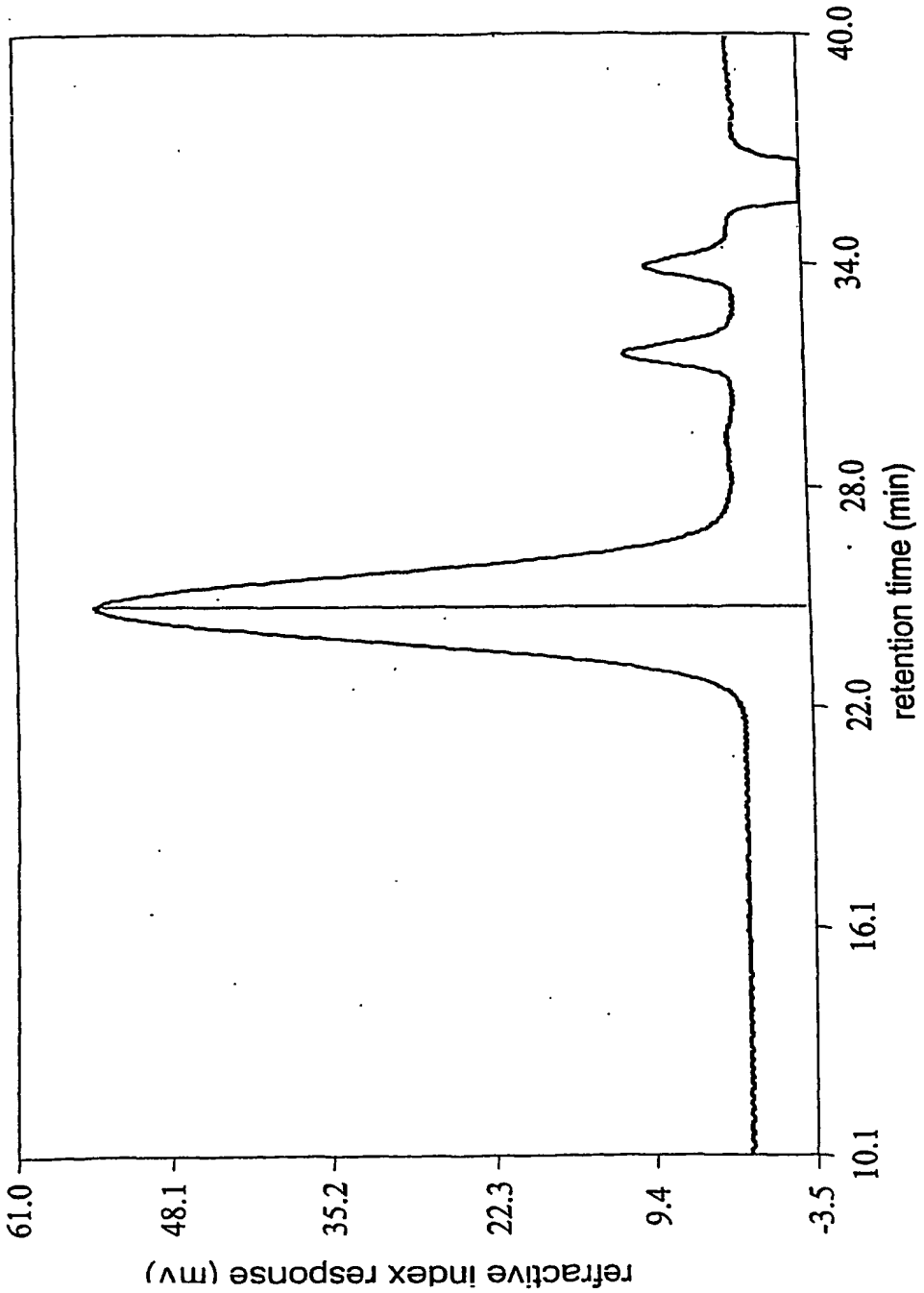

refractive index response (mv)
61.0
48.1
35.2
22.3
9.4
-3.5
10.1
16.1
22.0
28.0
34.0
40.0
retention time (min)

Figure 6b

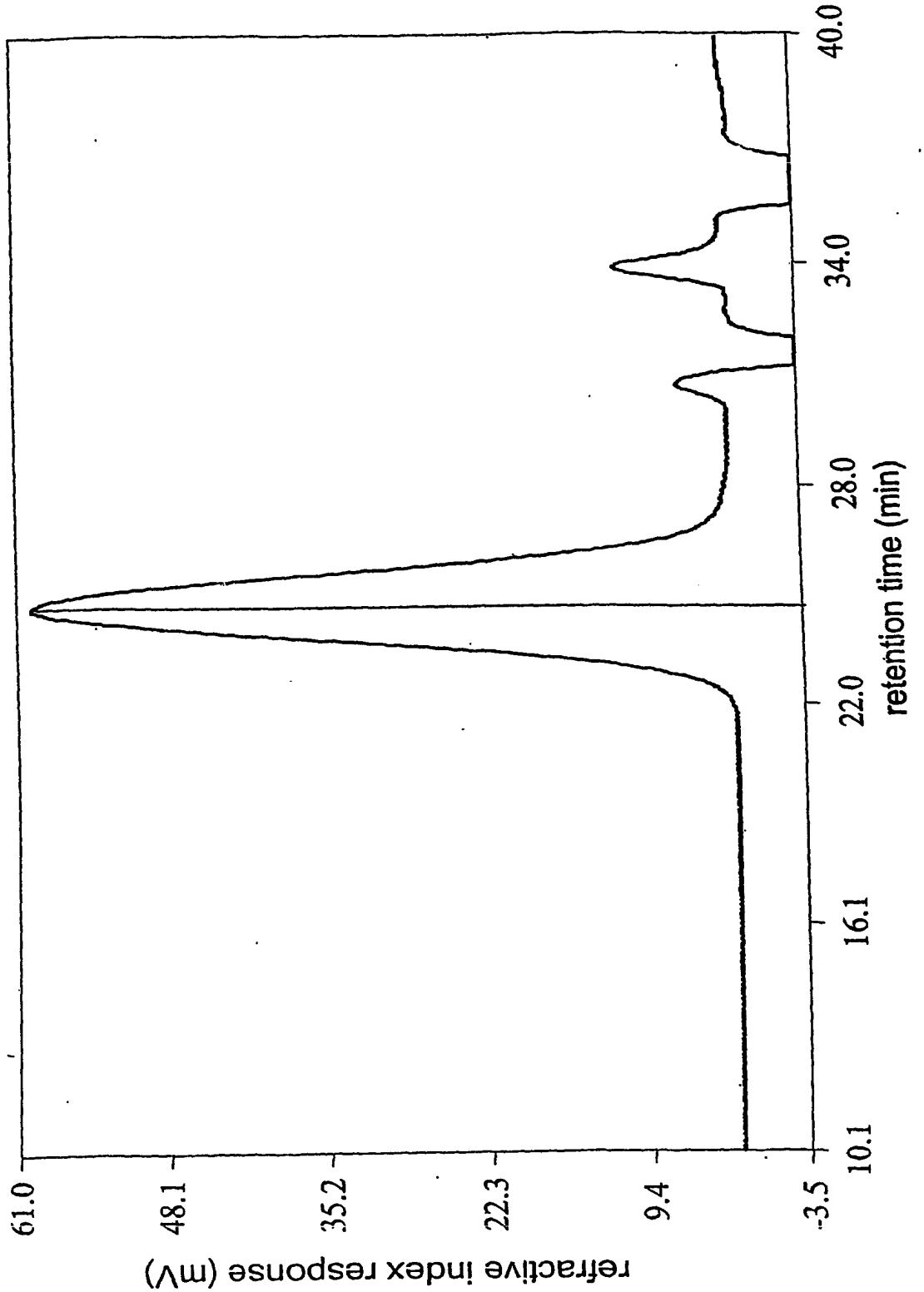

refractive index response (mV)
61.0
48.1
35.2
22.3
9.4
-3.5
10.1
16.1
22.0
28.0
34.0
40.0
retention time (min)

Figure 6c

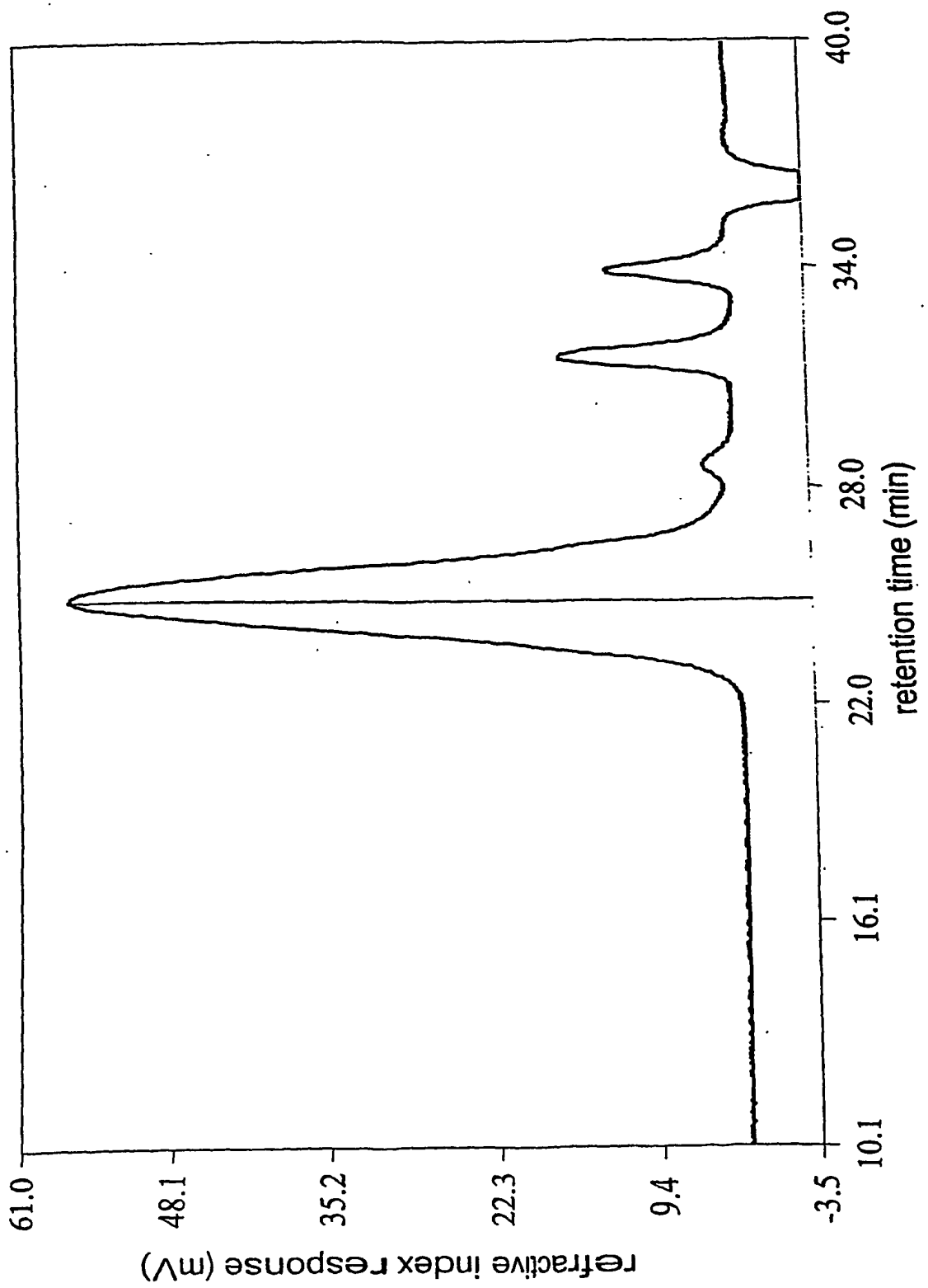

refractive index response (mV)
61.0
48.1
35.2
22.3
9.4
-3.5
10.1
16.1
22.0
28.0
34.0
40.0
retention time (min)

Figure 6d

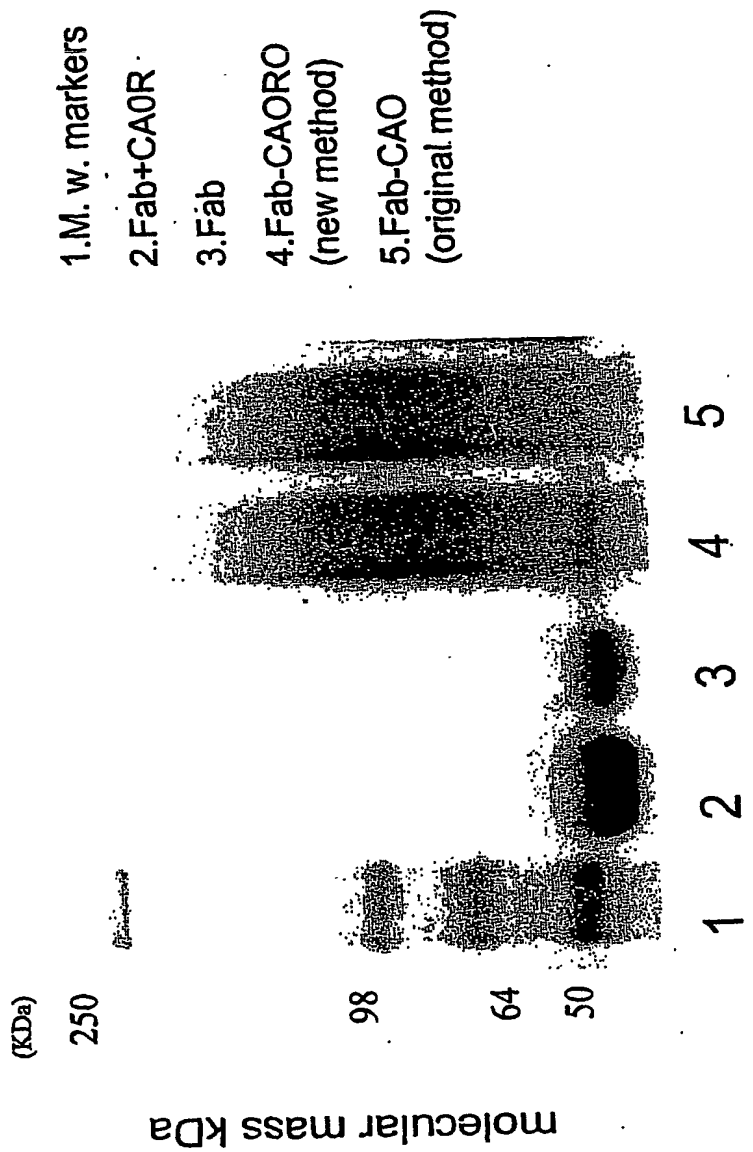
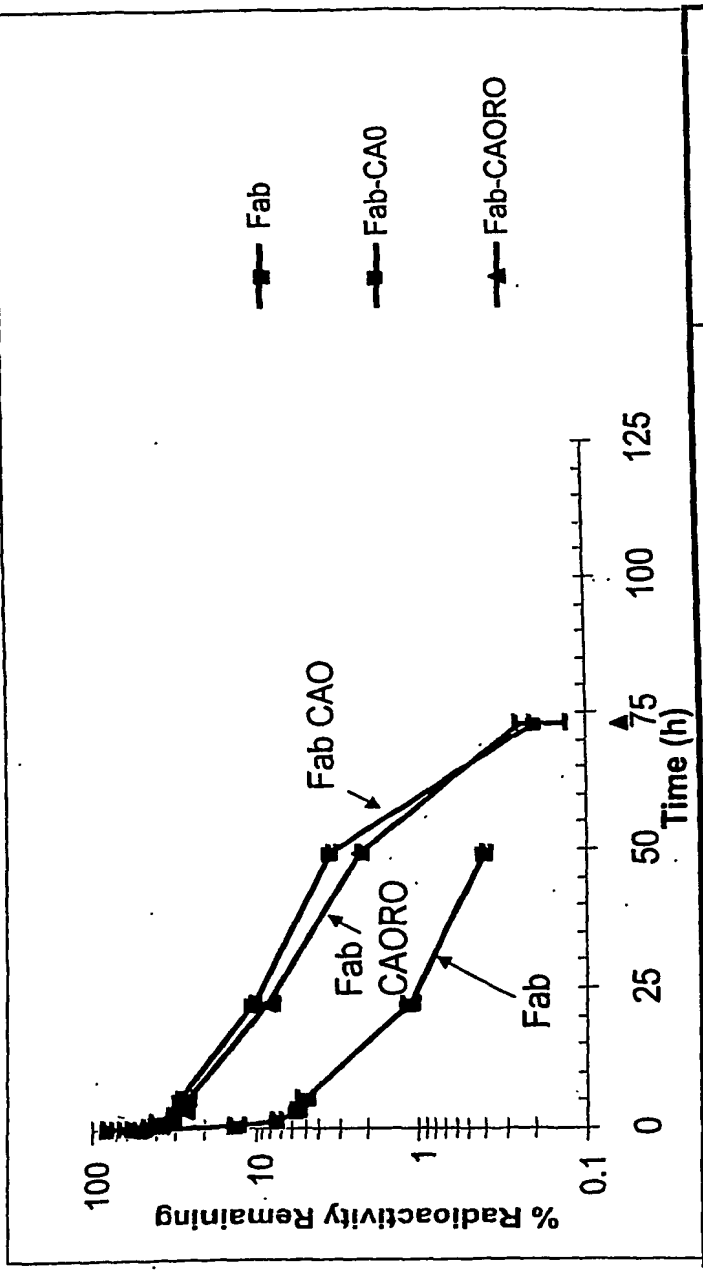

1.M. w. markers
2.Fab+CA0R
3.Fab
4.Fab-CAORO (new method)
5.Fab-CAO (original method)
(KDa)
250
98
64
50
1
2
3
4
5
molecular mass kDa

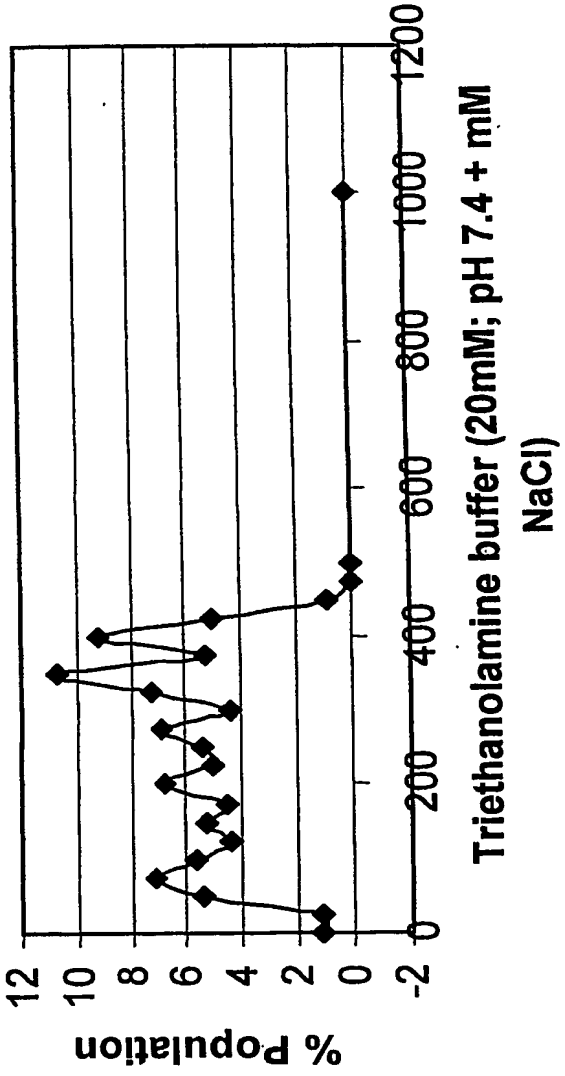
Figure 7

Figure 8



	Fab	Fab-conjugate prepared with CAO (original method)	Fab-conjugate prepared with CAORO (double oxidation method)
Area under curve	116	729	613

Figure 9


% Population
12
10
8
6
4
2
0
-2
0
200
400
600
800
1000
1200
Triethanolamine buffer (20mM; pH 7.4 + mM NaCl)

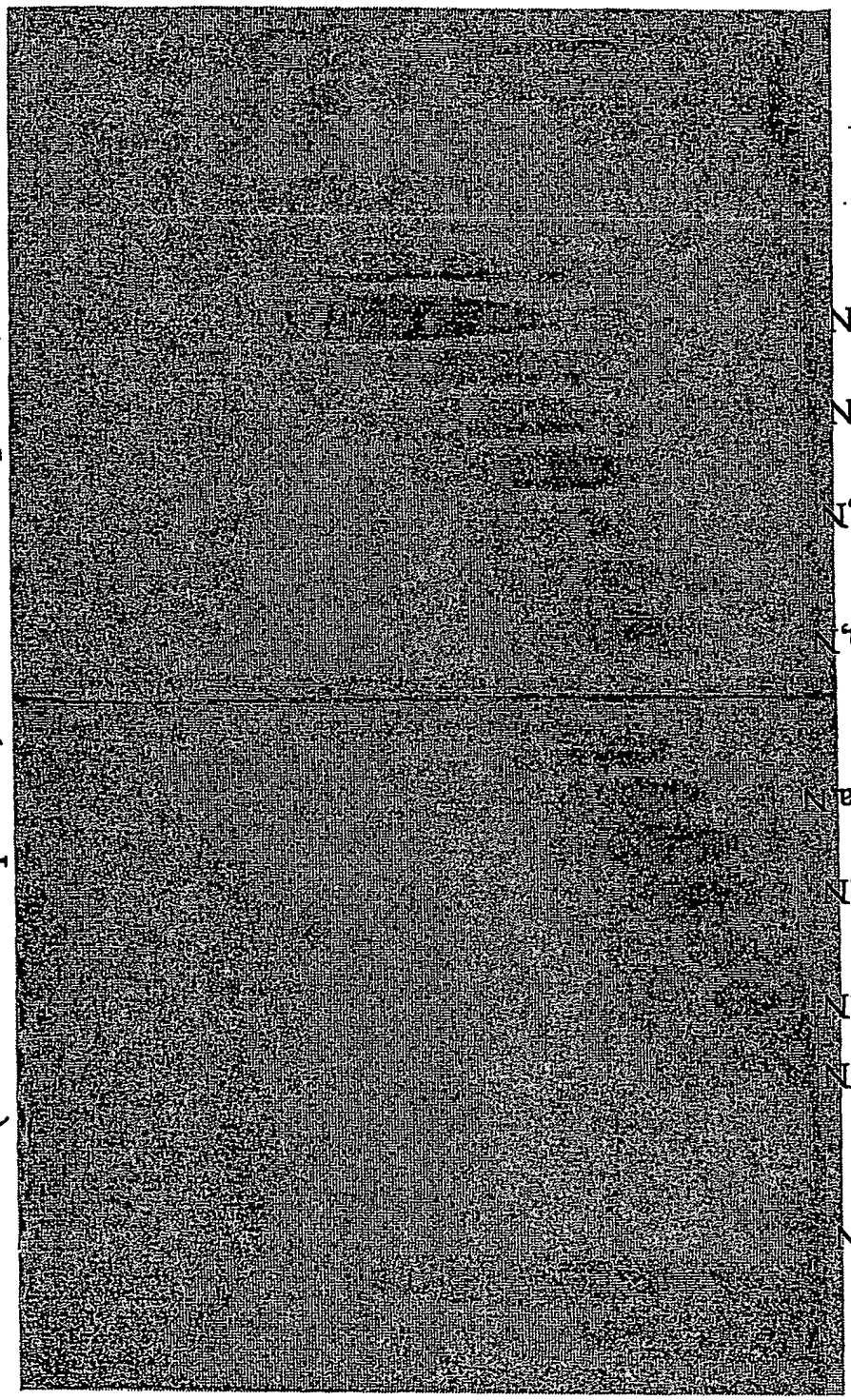


Figure 10: A typical native page of colominic acid fractions with m.w. (B = broad dispersed; N= narrow dispersed)

Figure 11

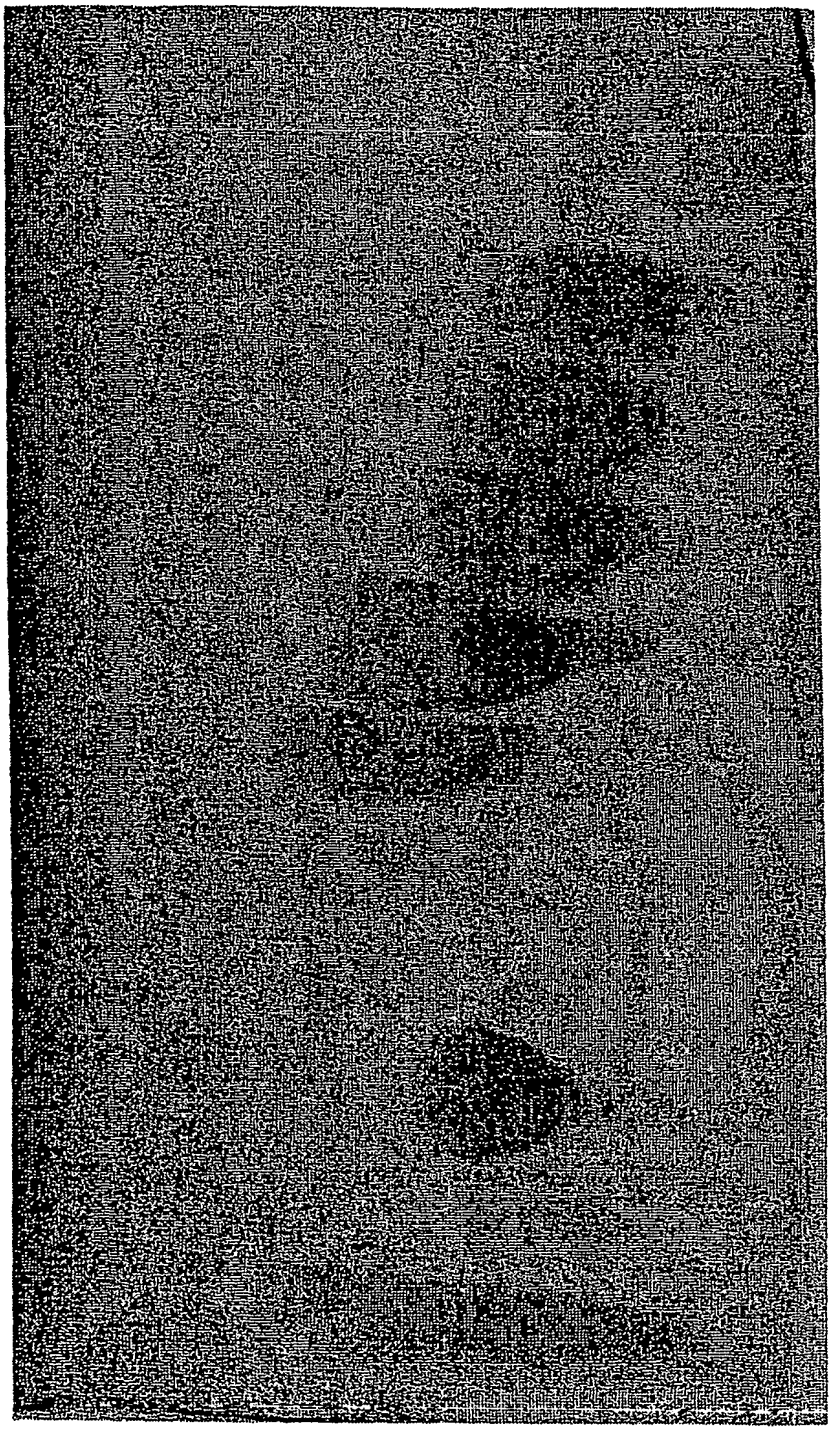

39K
22K
400II mM
450II mM
475 mM
450 mM
425 mM
400 mM
375 mM
350 mM
325 mM
11K

Figure 12: A typical GPC chromatogram for CA fractions

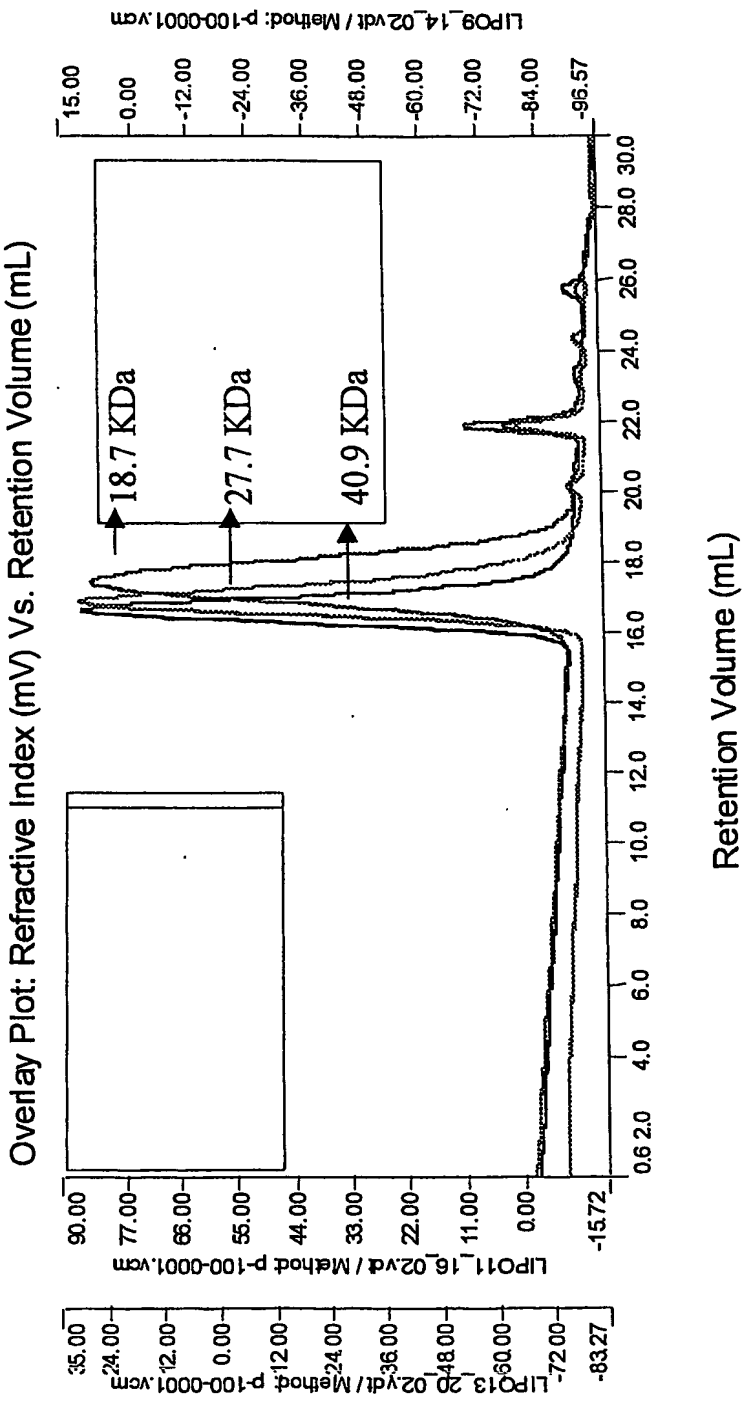


Figure 13 : Colominic acid samples from different steps of fractionation

1) 18.7 kDa disperse
2) 32.2 kDa from IEC
3) 32.2 kDa ultrafiltered
4) 32.2 kDa post oxidation
5) 40.9 KDa from IEC
6) 40.9 KDa ultrafiltered
7) 4.90 KDa post oxidation

1 2 3 4 5 6 7

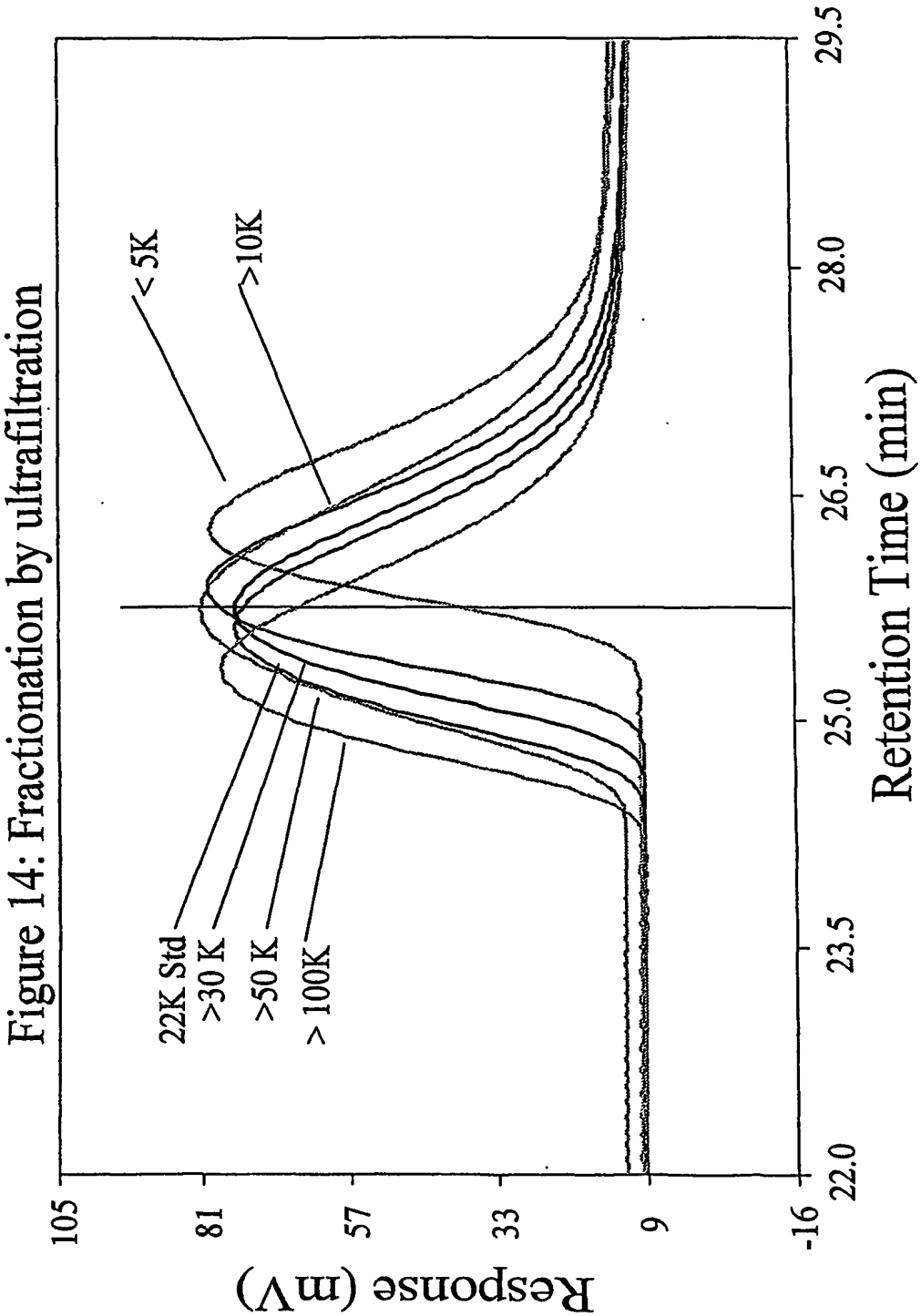


Figure 14: Fractionation by ultrafiltration
Response (mV)
105
81
57
33
9
-16
22.0
23.5
25.0
26.5
28.0
29.5
Retention Time (min)
< 5K
>10K
22K Std
>30 K
>50 K
> 100K

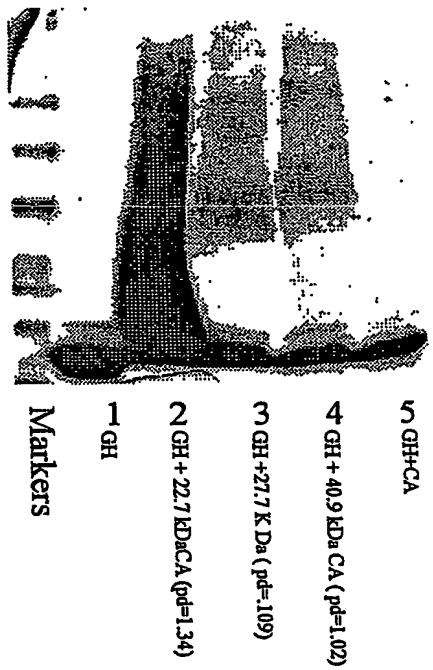


Figure 15

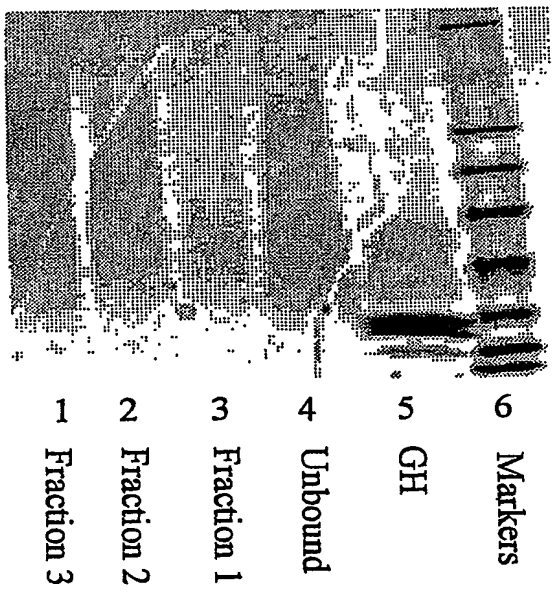


Figure 16

Figure 17

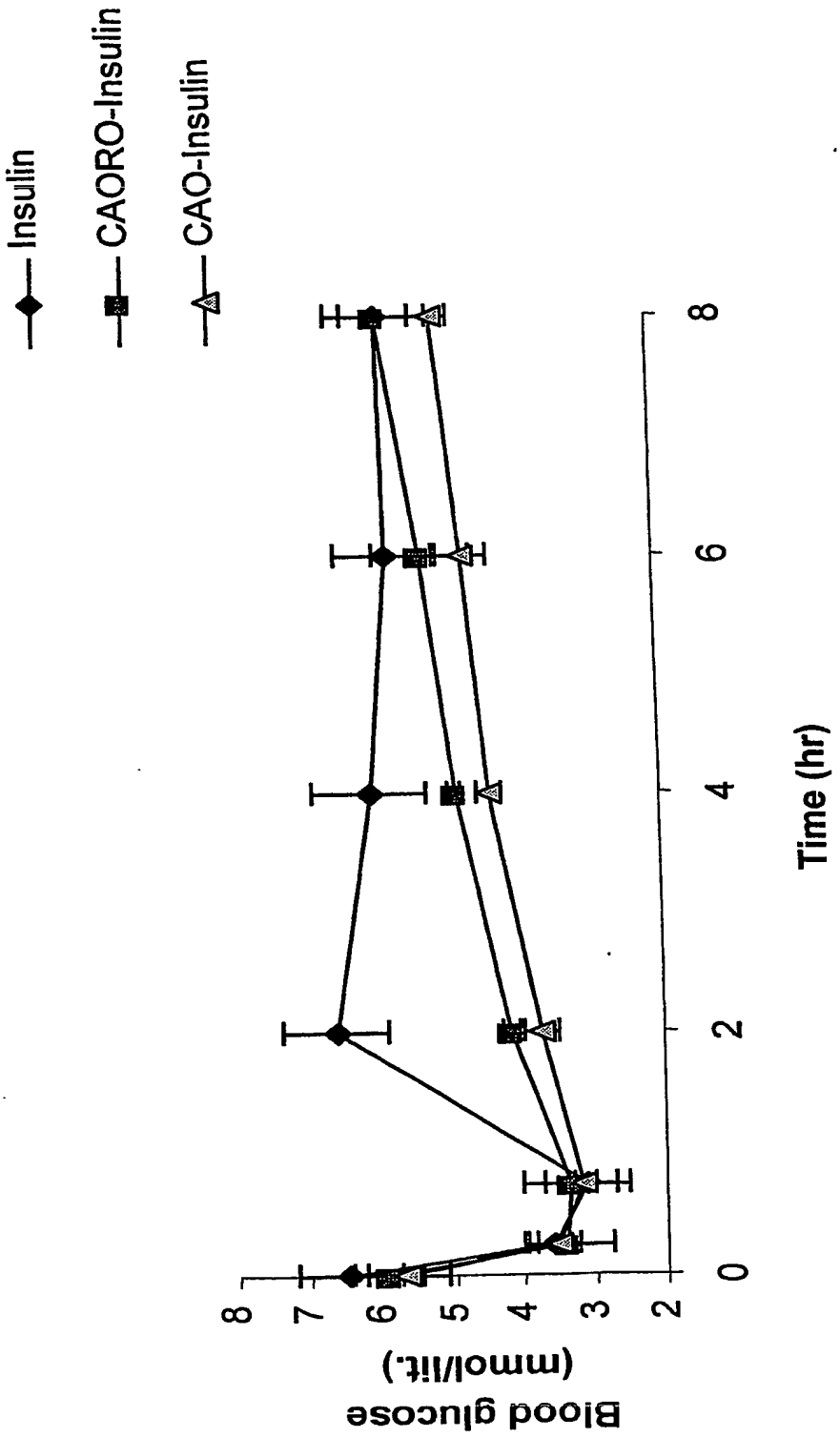

Insulin
CAORO-Insulin
CAO-Insulin
Blood glucose (mmol/lit.)
8
7
6
5
4
3
2
0
2
4
6
8
Time (hr)

Table 6: Ion exchange chromatography of CA22.7: Scale up (900 matrix)

Sample (in 20mM Triethanolmine buffer+mM NaCl, pH7.4)	M.W. (Pd) 12.5 g batch	M.W. (Pd) 25 g batch
350 mM	15490 (1.008)	10.470 (1.173)
375 mM	19960 (1.010)	24659 (1.019)
400 mM	25829 (1.019)	29573 (1.018)
425 mM	33763 (1.023)	34160 (1.011)
450 mM	46880 (1.058)	44400 (1.013)
475 mM		28500 (1.376)